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Fermentation of Biomass Hemicelluloses to Ethanol

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ABSTRACT

This article represents a compilation of current research endeavors and speculations in the chemical/biological processes employed in the conversion of biomass hemicelluloses (xylans) to sugar and subsequent fermentation. Current literature is reported on the generation of xylose hydrolyzates, pentose metabolism, alcohol production, and use of xylose isomerases. Summations on conversion processes to support a xylose fermentation at NRRC are discussed.

LITERATURE REVIEW

INTRODUCTION

Residues are the excesses and wastes from growing and processing raw agricultural products. The present and potential supply of agricultural byproducts from domestic crops is not known in the United States with certainty; however, the annual total certainly exceeds 800 million tons (dry basis). The feasibility of utilizing these residues for producing energy or chemicals depends on their characteristics: composition, availability, supply, current usage, value of the residue for other uses, and the economics of collection and storage.

An accurate, comprehensive analysis of the characteristics of most agricultural residues is not available; however, recent publications describe the properties of a number of important agricultural and related industrial wastes (1, 2). Comprehensive reviews of research done in the sixties and seventies on utilization of animal products and on animal waste management have been reported by Mann (3) and Loehr (4). Other reviews (5, 6, 7) are mainly concerned with either chemical or microbiological conversion to useful byproducts: feed supplements, methane, biopolymers, and chemical feedstocks.

Cellulose, our most abundant renewable resource is available as a renewable plant material from sources such as wood pulp, newsprint, urban waste, agri-residues, and manure. An estimated 22 X 10^9 tons of

cellulose is generated by photosynthesis annually worldwide (8). Only about 4 X 10^9 tons/yr (20%) is readily available for conversion to energy or feedstuffs.

Dry cellulosic materials (in average) have a heat value of about one-third that of hydrocarbon of equal weight. Therefore, cellulosic materials can be looked at seriously in terms of renewable, industrial resource. One alternative to the conversion of cellulose is the production of alcohol. Alcohol can be burned in internal combustion engines and can be used in solvents, in beverages, and in hydrocarbon synthesis (via ethylene). The basic steps in processing cellulose to alcohol include conversion of cellulose to glucose, followed by microbial fermentation to alcohol and recovery of alcohol by distillation.

Several chemical and biological processes have been used to modify the abundant lignocellulosic byproducts of agricultural and wood-processing industries to increase their digestibility for animals (9, 10).

Cellulose/Hemicellulose Hydrolysis

Most plant fibers contain cellulose, hemicellulose, and lignin in approximate ratios of 4:3:3 (11). Cellulose is a homogeneous polymer of glucose, whereas hemicellulose molecules are often polymers of pentoses (xylose and arabinose), hexoses (mannose), and a number of sugar acids. Lignin, a polyphenolic macromolecule (12) is relatively high in C and H and lower in O content than are cellulose and hemicellulose, and it has the highest heat value of the three (13). Hydrolysis of hemicellulose to mono- and oligosaccharides can be accomplished with either acids or enzymes under moderate conditions (14, 15). Unlike hemicellulose, cellulose is resistant to hydrolysis. Cellulose fibers generally consist of a highly ordered crystalline structure of cellulose surrounded by a lignin seal, which becomes a physical barrier to easy hydrolysis. The easily hydrolysable portion of cellulose (amorphous region) is about 15% and the remainder, the resistant residue, is crystalline cellulose. Crystalline cellulose may be hydrolyzed by strong acid, but this also causes degradation of the glucose monomer.

Hemicellulose is one of the major components of renewable resources, comprising upwards of 35% of the plant material (16). Hydrolysis of hemicellulose yields a mixture of sugars, primarily D-xylose as the major component. For complete utilization of biomass-derived sugars in the production of fermentation alcohol, the conversion of both the cellulosic and hemicellulosic components is warranted. Until recently, no yeasts were reported to ferment pentoses, although some were capable of aerobic metabolism (17). Hemicellulose-derived pentoses can be obtained easily in good yield from residues using a relatively simple process (15). Xylan can be degraded by yeast of the genera Aureobasidium, Cryptococcus, and Trichosporon and Candida utilis and Candida albicans utilize D-xylose (18).

Clostridium thermosaccharolyticum ferments xylose to a mixture of ethanol, lactic acid, and acetic acids (19). Fusarium oxysporam and other Fusarium species degrade xylose and have been used in combination with the yeast Saccharomyces cerevisiae (20, 21).

FERMENTATION OF PENTOSES

Hemicellulose-derived pentoses can be produced from a variety of available

residues in reasonable yields by means of dilute acid hydrolysis at moderate temperatures. Using relatively simple chemical processes, pentoses may be the least expensive carbohydrates available for fermentation to alcohol and other feedstock chemicals. An alcohol from hemicellulose process could be incorporated into a cellulosic industry as an initial step for utilization of pentose sugars. Initial processing of the 5-C sugars could be coupled to the cellulose hydrolysis for more efficient utilization of biomass to fuels.

The major drawback to the application of these concepts was the inability of yeasts to convert pentose sugars to ethanol and lack of strong physiological evidence relative to ethanol production from complex sugar hydrolyzates. To fully utilize biomass-derived sugars in the production of ethanol, fermentation of the hemicellulose-derived pentoses is important.

All pentose-fermenting, ethanol-producing bacteria so far studied appear to use a combination of the pentose-phosphate and Embden-Meyerhoff pathways for conversion of pentulose phosphates to pyruvic acid (22). Amongst the fungi, only members of the genus Fusarium have been reported to produce ethanol from pentose (23).

Bacteria such as Aeromonas hydrophilia (24), Clostridium thermosaccharolyticum (19), and Klebsiella pneumoniae (24) yield mixtures of fermentation products, including ethanol, from D-xylose.

Recently Chiang, Gong and coworkers (25, 26) have reported on ethanol production from D-xylose in the presence of D-xylose-isomerizing enzyme. D-xylulose, an intermediate of D-xylose catabolism, was observed to be fermentable to ethanol and CO_2 in a yield of greater than 80% by bakers' yeast. Similar observations have also been reported by Wang et al. (27, 28) on isomerase catalyzed conversion of xylose to xylulose followed by fermentation to ethanol. Wang and coworkers (29) have also recently published on a number of yeasts capable of D-xylulose catabolism in the presence of air and growth.

Recent findings (30-32) have demonstrated a yeast system capable of direct fermentation of xylose to ethanol. This organism, Pachysolen tannophilus ferments glucose and xylose to alcohol in the presence of oxygen. Maleszka et al. (33) showed enhanced rates of ethanol production by P. tannophilus from xylose with recycled or immobilized cells. Fermentation under these conditions did not require aeration. Slininger and coworkers (34) demonstrated continuous fermentation of xylose by P. tannophilus entrapped in calcium alginate beads. Cultures retained some 50% of their initial productivity after 26 days operation. Yields of 0.35 g ethanol/g xylose were obtained. Recent findings (35) demonstrate a Candida tropicalis capable of xylose conversion to ethanol under aerobic conditions.

Since a xylose isomerase has not been identified in P. tannophilus cells, it would appear that another route is functional to metabolize the xylose. Smiley (36) has recently demonstrated the presence of a NADP-dependent xylose reductase and NAD-dependent xylitol dehydrogenase in xylose grow P. tannophilus cells. These two enzymes would catalyze the formation of xylulose which could be phosphorylated to xylulose-5-PO_4 and metabolized through the pentose cycle to ethanol. Further documentation of this pathway in the physiology of P. tannophilus is warranted.

The demonstration of microbial systems for direct fermentation of glucose and xylose to ethanol provides a new dimension in the conversion of biomass to liquid fuels, especially ethanol. Processes for the conversion of cellulose and hemicellulose to ethanol could be integrated into existing cellulose-conversion plants where the hemicellulose-derived pentoses are underutilized. Future conversion industries would incorporate both the grain- and residue- derived sugars for optimum production of fermentation alcohol. Glucose liquors from acid or enzyme hydrolyzed cellulosics would provide a substrate for a Saccharomyces fermentation that would be coupled to conversion of xylose and xylans to ethanol by other yeasts.

FERMENTATION OF XYLOSE AND WHEAT STRAW HEMICELLULOSE HYDROLYZATES

Batch fermentations in our laboratory (31, 34) with Pachysolen tannophilus initially containing 50 g/liter D-xylose yielded 0.34 g of ethanol per gram of pentose consumed. Aerobic conditions were required for cell growth but not for ethanol production. Both alcohol formation and growth were optimum when incubation temperature was 32°C, when pH was near 2.5, and when D-xylose and ethanol concentrations did not exceed 50 g/liter and 20 g/liter, respectively.

The most recent information from our laboratory represents research findings on the production of glucose and xylose from straw and subsequent direct fermentation of both sugars to ethanol.

Agricultural straw was subjected to thermal or alkali pulping prior to enzymatic saccharification. When wheat straw (WS) was treated at 170°C for 30 to 60 min at water-to-solids ratio of 7:1, the yield of cellulosic pulp was 70 to 82% (Table 1). A sodium hydroxide extraction yielded a 60% cellulosic pulp and a hemicellulose fraction available for fermentation to ethanol (Table 2). The cellulosic pulps were subjected to cellulase hydrolysis at 55°C for production of sugars to support a C-6 fermentation. Hemicellulose was recovered from the liquor filtrates by acid/alcohol precipitation followed by acid hydrolysis to xylose for fermentation.

Pachysolen tannophilus strain NRRL 2460 is capable of an ethanol fermentation of xylose under initial aeration conditions for generation of high cell populations (31). Detroy et al. (32) have reported batch fermentations with 70 g/liter D-xylose resulting in 0.3 g ethanol/g of pentose metabolized in 6 days. Figure 1 depicts the replication cycle of P. tannophilus at 25°C on 7% xylose. Cell populations double every 24 hr for approximately 3 days. Ethanol concentrations of 2.0% were achieved by 6 days with complete utilization of the xylose available.

Our most recent experiments with P. tannophilus involve fermentation of xylose from crude WS hydrolyzates. The yeast produced 7.2 g (0.72%) ethanol from a hydrolyzate containing 43 g/liter xylose (4.3%) as shown in Figure 2. All of the xylose was consumed within 4 days with only a slight effect upon cell replication. The sub-optimal yield for ethanol is probably due to the presence of lignin byproducts and degraded sugar derivatives in the concentrated WS hydrolyzates. Further optimization of both the processing of hydrolyzates and the fermentation are underway in our laboratory.

Figure 3 depicts an overall schematic with an initial chemical pretreatment to yield a xylose/pentosan component plus the cellulosic residue for ethanol production. From 500 g of WS, one obtains 400 g cellulosic pulp after chemical pretreatment with 4% NaOH for 6 hr (37). The liquor

TABLE I. CHARACTERISTICS OF THERMALLY PULPED WHEAT STRAW (30 MIN AT 170°C)

		Cellulosic pulp composition			
	Residue yield	Cellulose M.E.A.	Pentosans	Lignin	Ash
	%	%	%	%	%
	82.5	46.3 (33)[d]	18.0 (29)	16.5 (14)	7.68 (9)

		Hydrolyzate composition				
	Yield[a]	Nitrogen[b]	Pentosans	Lignin	Ash	Xylose[c]
pH	%	%	%	%	%	%
4.2	11.3	0.57	35.3	11.1	17.4	23.2

[a]Solids, basis original wheat straw. Free liquor collected through condenser.

[b]Kjeldahl method.

[c]After dilute acid hydrolysis.

[d]Values in parentheses represent analysis values for untreated WS.

TABLE II. CHARACTERISTICS OF ALKALI-EXTRACTED WHEAT STRAW

	(Cellulosic Pulp)			
	Composition			
Residue[a] yield %	Cellulose M.E.A. %	Pentosans %	Lignin %	Ash %
60.3	54.3	20.9	8.34	5.90

(Hemicellulose ppt. from WS)		
Yield %	Lignin %	Xylose[b] %
18.3	13.1	18.8

[a]Extracted overnight at room temperature with 4% sodium hydroxide solution.

[b]After dilute acid hydrolysis.

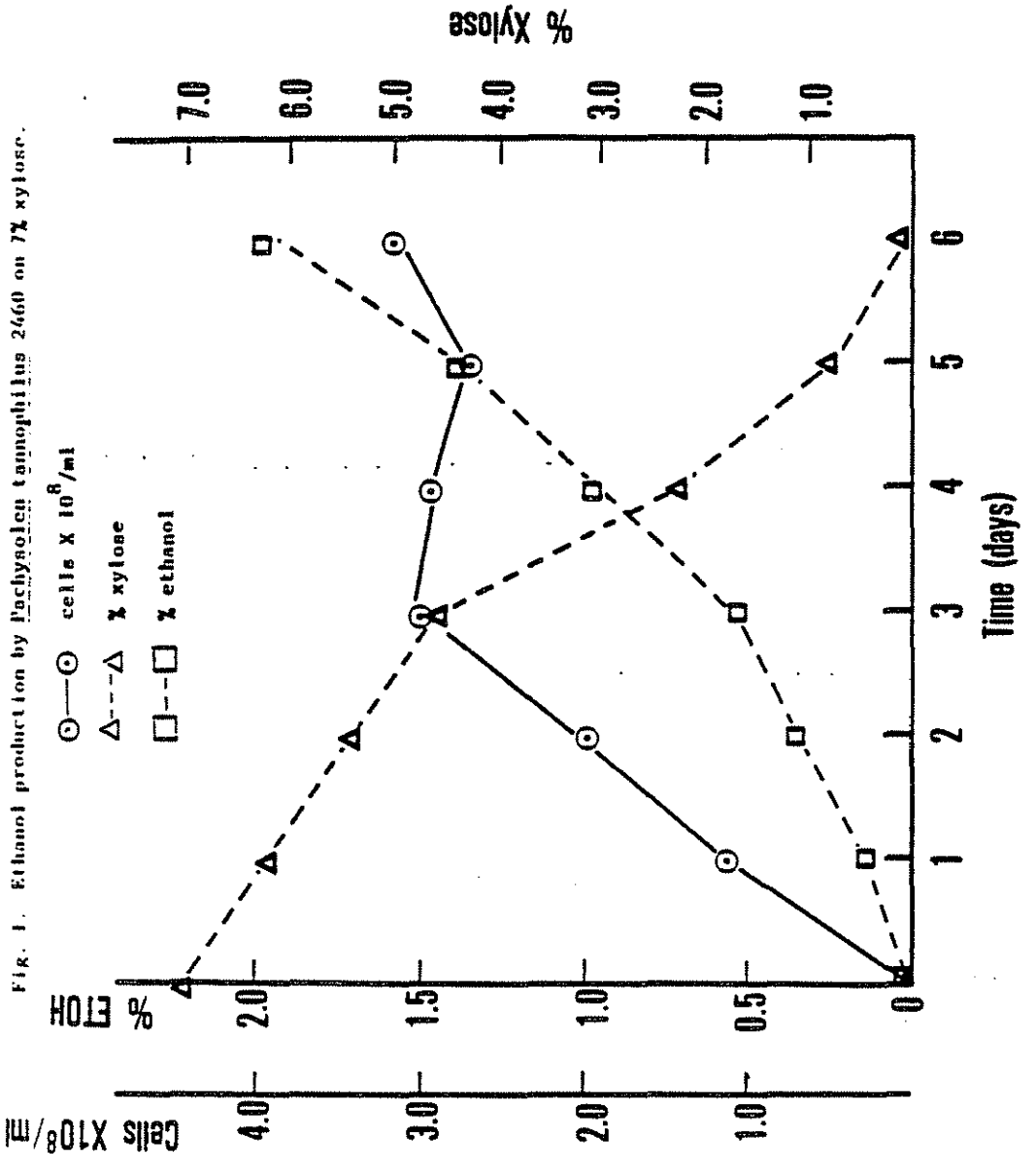
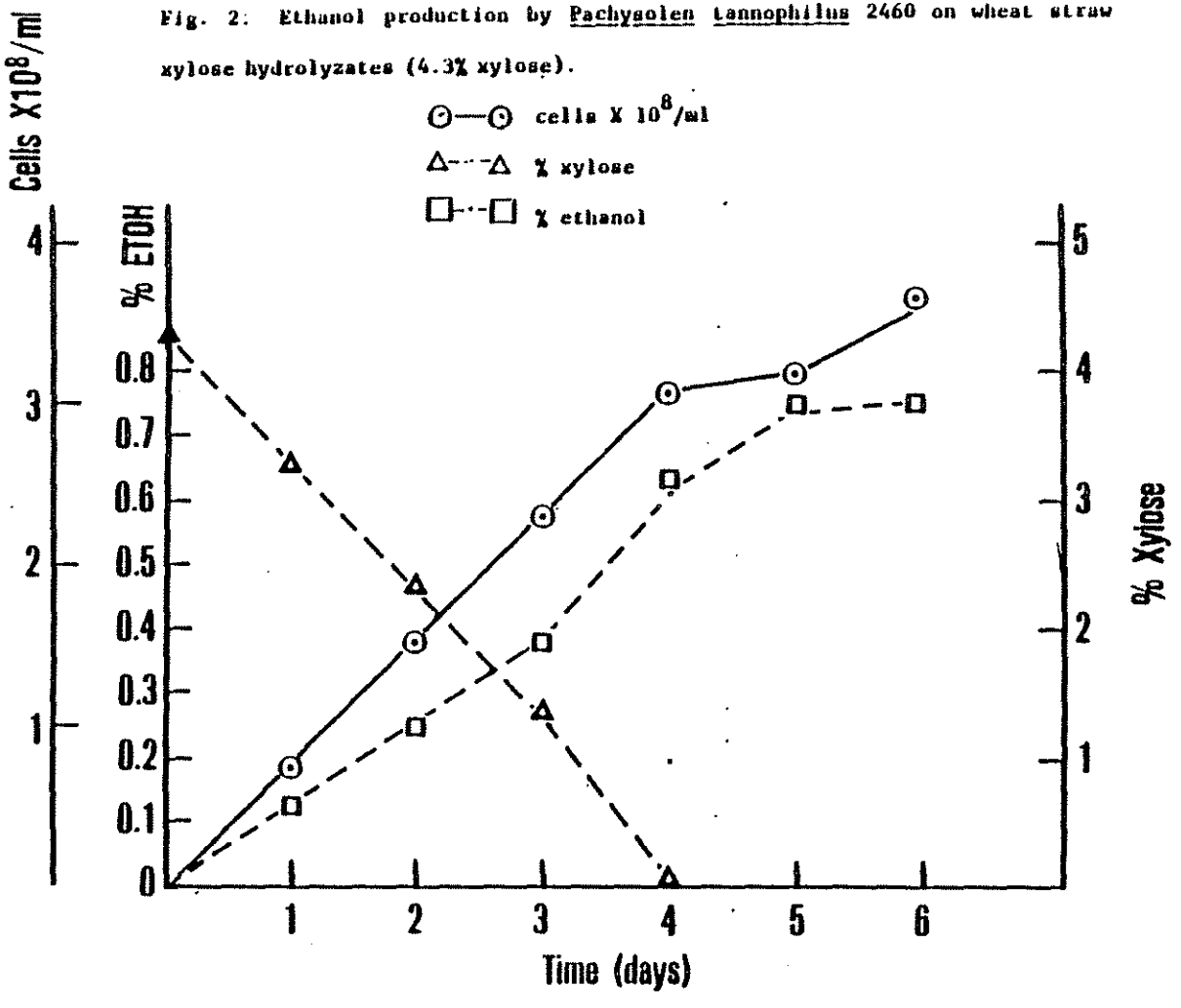


FIG. 1. Ethanol production by Pachysolen tannophilus 2460 on 7% xylose.

Fig. 2: Ethanol production by Pachysolen tannophilus 2460 on wheat straw xylose hydrolyzates (4.3% xylose).



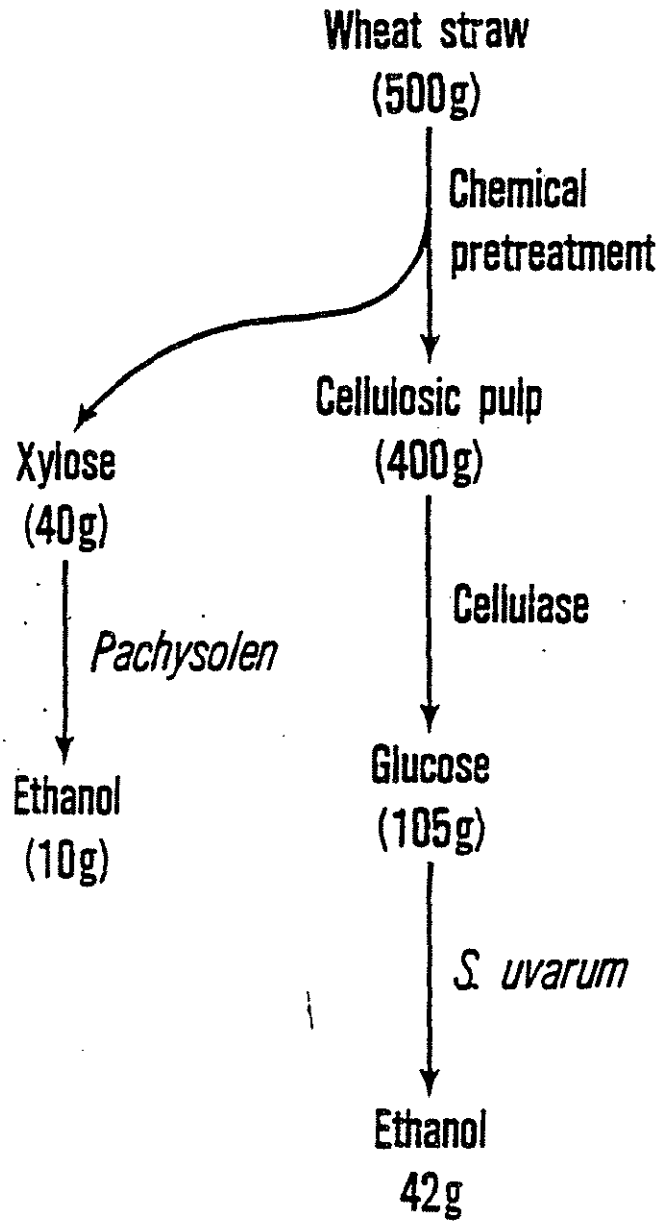


Fig. 3. Fermentation schematic for ethanol from wheat straw.

contains some 40 g of fermentable D-xylose which, although sub-optimal, supports the P. tannophilus 5-C fermentation treatment of the cellulosic pulp with cellulase (10 IU/g) for 6 hr yields 105 g of fermentable sugar, which is only 60% of the available glucose in the pulp. Addition of S. uvarum cells to the saccharified material yields 42 g ethanol (80% of theoretical amount) in 48 hr.

Although the final yields of sugars from WS residues is not optimal, fermentation of the xylose and glucose produced in the aforementioned processes has been achieved with S. uvarum and P. tannophilus. With the advent of cultures such as the Pachysolen yeast direct, total fermentations of residue polysaccharides to ethanol can be optimized.

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